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Micellar versus hydro-organic mobile phases for retention-hydrophobicity relationship studies with ionizable diuretics and an anionic surfactant

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Abstract

Logarithm of retention factors ($\log k$) of a group of 14 ionizable diuretics were correlated with the molecular ($\log P_{o/w}$) and apparent ($\log P_{app}$) octanol–water partition coefficients. The compounds were chromatographed using aqueous–organic (reversed-phase liquid chromatography, RPLC) and micellar–organic mobile phases (micellar liquid chromatography, MLC) with the anionic surfactant sodium dodecyl sulfate (SDS), in the pH range 3–7, and a conventional octadecylsilane column. Acetonitrile was used as the organic modifier in both modes. The quality of the correlations obtained for $\log P_{app}$ at varying ionization degree confirms that this correction is required in the aqueous–organic mixtures. The correlation is less improved with SDS micellar media because the acid–base equilibriums are shifted towards higher pH values for acidic compounds. In micellar chromatography, an electrostatic interaction with charged solutes is added to hydrophobic forces; consequently, different correlations should be established for neutral and acidic compounds, and for basic compounds. Correlations between $\log k$ and the isocratic descriptors $\log k_w$, $\log k_{wm}$ (extrapolated retention to pure water in the aqueous–organic and micellar–organic systems, respectively), and φ_0 (extrapolated mobile phase composition giving a $k = 1$ retention factor or twice the dead time), and between these descriptors and $\log P_{app}$ were also satisfactory, although poorer than those between $\log k$ and $\log P_{app}$ due to the extrapolation. The study shows that, in the particular case of the ionizable diuretics studied, classical RPLC gives better results than MLC with SDS in the retention hydrophobicity correlations.

Keywords: Micellar liquid chromatography; Hydrophobicity; Octanol–water partition coefficients; Mobile phase composition; Retention hydrophobicity relationships; Diuretics; Sodium dodecyl sulfate

1. Introduction

The hydrophobic character of a molecule is an important physico-chemical property in relation to its biological activity. It is a parameter difficult to quantitate. The most widely accepted hydrophoby value is the octanol–water distribution constant scale. This constant is defined as the ratio of the concentrations of the solute in the two phases of a saturated 1-octanol–water system [1,2]. Direct measurement of the octanol–water distribution constant, $K_{D_{o/w}}$, still commonly called partition coefficient, a term not recommended by IUPAC, and noted $P_{o/w}$, has usually been made by the traditional shake-flask method. This is a tedious and problematic procedure that leads to poor inter-laboratory reproducibility. For this reason, aqueous–organic reversed-phase liquid chromatographic (RPLC) procedures have grown in use for the estimation of $P_{o/w}$, since hydrophobic forces usually dominate the retention of solutes. Furthermore, RPLC permits to obtain easily many reproducible data, needs only a small amount of sample and the impurities do not interfere since they are separated. There are many examples in the literature of the successful use of RPLC in predicting $P_{o/w}$ for a wide range of compounds, using either isocratic or gradient elution [3–11]. Other chromatographic parameters, obtained by extrapolation of the retention data from various aqueous–organic mobile phase compositions, have also been used as a measure of hydrophobicity. Two examples are the common isocratic descriptors $\log k_w$ (extrapolated retention to pure water), and φ_0 (extrapolated mobile phase composition giving a retention factor of unity ($\log k = 0$) or retention twice the dead time) [12–14].

The hydrophobicity measurements using RPLC are not perfect. For example, the extrapolated k_w values

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depend on the organic modifier used. These problems are mainly due to the activity of residual silanols and other non-partitioning retention mechanisms with alkyl-bonded stationary phases [12]. In order to avoid these practical difficulties, octanol-saturated columns [5] or alternative liquid chromatographic techniques, such as counter-current chromatography (CCC) [15–18] or micellar liquid chromatography (MLC) [19–26], which allow the direct and indirect measurement of $P_{o/w}$, have been suggested. MLC has attracted some attention due to several interesting reported examples, where the retention correlated very satisfactorily with diverse biological descriptors [20–23,25].

The success of the correlations in any chromatographic mode depends on the quality of $\log P_{o/w}$ data, especially when working with compounds exhibiting acid–base behavior. Many natural compounds contain one or several ionizable groups, and a small pH change in solution can induce a large change in the relative concentration of acid–base species. The measured $P_{o/w}$ depends thus strongly on the ionization state of the solute, and consequently, non-linear relationships of $\log k$, $\log k_w$ and φ_0 are expected with $\log P_{o/w}$, unless $P_{o/w}$ values of the molecular and ionic forms are available.

CCC was proved to be a powerful tool able to provide accurate $P_{o/w}$ data [15–18]. In this technique, stationary and mobile phases are two non-miscible liquids. The partition coefficients are obtained with no approximation since the two liquid phases (e.g. octanol and water) are used in the CCC apparatus exactly as they are in the shake-flask method [27,28]. Changing the pH of the aqueous phase, CCC allows measurement of apparent $P_{o/w}$ values (P_{app}). For acidic compounds of the AH-A^- type, P_{app} varies with pH as follows:

$$P_{app} = \frac{P^0_{o/w} + P^-_{o/w}(K_a/h)}{1 + (K_a/h)} \quad (1)$$

and for basic compounds of the BH^+-B type, the relation is:

$$P_{app} = \frac{P^0_{o/w} + P^+_{o/w}(h/K_a)}{1 + (h/K_a)} \quad (2)$$

where $P^0_{o/w}$, $P^-_{o/w}$ and $P^+_{o/w}$ are the octanol–water partition coefficients of the molecular, anionic and cationic species, respectively. K_a is the acid–base dissociation constant and h the concentration of hydrogen ions [16].

MLC solute retention depends on the type of interaction (electrostatic and/or hydrophobic) with the micelles and surfactant-modified stationary phase [29–31]. Non-ionic solutes should only be affected by hydrophobic interactions, and give rise to acceptable linear relationships of $\log k$ versus $\log P_{o/w}$ at low micellar concentrations [32–34]. However, for highly hydrophobic compounds and large micelle concentrations, deviations from linearity can result [35,36]. For charged solutes, electrostatic interactions are established in addition to the hydrophobic forces, producing two different situations: if charges on solute and surfactant are of the same sign, the electrostatic repulsion between solute and the surfactant-modified stationary phase will decrease the retention and the solute will elute at short retention times, even with the void volume. If charges are opposite, electrostatic attraction will increase the retention, even for highly polar compounds.

This work studies the relationships between both RPLC and MLC (with sodium dodecyl sulfate, SDS) retention data sets and some derived hydrophobic descriptors with $\log P_{o/w}$ data measured by CCC in aqueous phase, for a group of ionizable compounds of different polarity and acid–base properties. Several diuretics with a wide variety of chemical structures, bearing ionizable groups and different protonation constants, have been selected as probe compounds.

2. Experimental

2.1. Reagents

Aqueous–organic and micellar–organic mobile phases were prepared with acetonitrile (HPLC grade, Scharlab, Barcelona, Spain). For the micellar mode, only SDS (99% purity, Merck, Darmstadt, Germany) was used. In both cases, pH was adjusted to 3, 4, 5 and 7 with 0.1 M citric acid (Prolabo, Paris, France) and 1 M NaOH (Probus, Badalona, Spain), after adding the organic solvent. Acetonitrile concentration in the mobile phases is given as volumetric fraction (%, v/v).

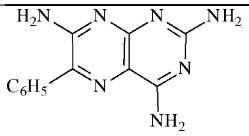
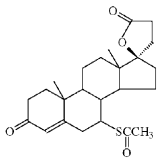
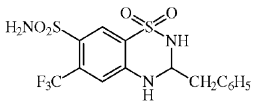
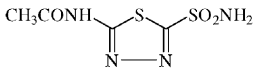
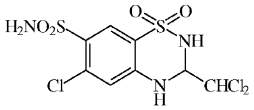
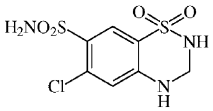
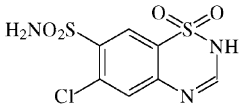
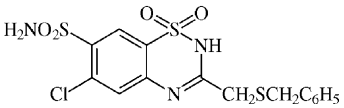
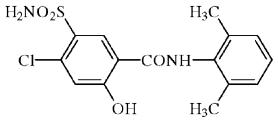
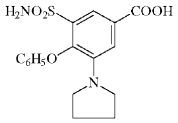
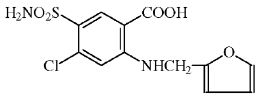
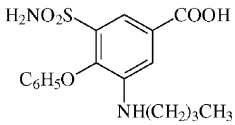
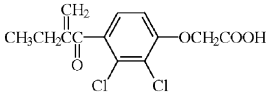
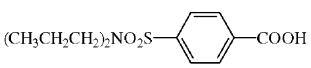
Stock standard solutions of 100 μg/ml acetazolamide (Lederle, Madrid, Spain), benzthiazide, bumetanide, chlorothiazide, furosemide, hydrochlorothiazide, probenecid, triamterene and trichloromethiazide (Sigma, St. Louis, MO, USA), bendroflumethiazide (Davur, Madrid), ethacrynic acid (Merck, Sharp & Dohme, Madrid), piretanide (Cusi, Barcelona), spironolactone (Searle, Madrid), and xipamide (Lacer, Sardenya, Barcelona), were prepared. The diuretics, except those of Sigma, were kindly donated by the pharmaceutical laboratories. Table 1 shows their structures. The compounds were dissolved in a few milliliters of ethanol (Prolabo), with the aid of an ultrasonic bath (Model 617, Selecta, Barcelona). Working solutions were diluted to 20 μg/ml with water and 0.1 M SDS for the aqueous–organic and micellar–organic mode, respectively. Furosemide and trichloromethiazide solutions were protected from light with aluminium foil. All solutions were kept in the dark at 4 °C.

2.2. Apparatus

The HPLC system consisted of an Agilent chromatograph (Palo Alto, CA, USA), with a HP 1100 isocratic pump, an autosampler with 2 ml vials, and a HP 1050 UV-vis detector. Data acquisition was obtained through the Peak 96 software.

The analytical separation with both aqueous–organic and micellar–organic mobile phases was accomplished using a

Table 1
Structures, dissociation constants and octanol–water partition coefficients of the studied diuretics

Compound	Structure	pK_a [a]	log $P^{o}_{o/w}$ [b]	log $P^{ion}_{o/w}$ [b,c]
Triamterene		6.2	1.22	$-\infty$ (BH^+)
Spironolactone		Non ionizable	2.71	Non ionizable
Bendroflumethiazide		9.0	1.95	0.057 (A^-)
Acetazolamide		7.4	0.30	0.96 (A^-)
Trichloromethiazide		10.6, 8.6, 7.3	1.00	0.15 (A^-)
Hydrochlorothiazide		7.0	0.11	0.18 (A^-)
Chlorothiazide		6.7	0.35	$-\infty$ (A^-)
Benzthiazide		6.0	1.73	$-\infty$ (A^-)
Xipamide		10.0, 4.8	2.19	2.00 (A^-)
Piretanide		4.1	2.20	1.70 (A^-)
Furosemide		7.5, 3.8	1.81	1.40 (A^-)
Bumetanide		7.7, 3.6	2.09	0.70 (A^-)
Ethacrynic acid		3.5	2.20	0.44 (A^-)
Probenecid		3.4	1.40	$-\infty$ (A^-)

[a] Refs. [37,38].
[b] Ref. [16] ($P^{0}_{o/w}$ and $P^{ion}_{o/w}$ are octanol–water partition coefficients of the molecular and ionic species, respectively).
[c] Log $= -\infty$ means $P^{ion}_{o/w} = 0$.

single Kromasil C_{18} column (5 μm particle size, 125 mm × 4.6 mm i.d., Análisis Vínicos, Ciudad Real, Spain) that was connected to a 30 mm guard precolumn of similar characteristics (Scharlab). The flow-rate was 1.0 ml/min, and the injection volume, 20 μl. The chromatographic runs were carried out at room temperature. The diuretics were monitored at 274 nm. Duplicate injections were made. The dead time was determined as the mean value of the first significant deviation of the base-line in the chromatograms. After working with a micellar phase, the Kromasil C_{18} column was rinsed with the sequence pure water, pure methanol and 70% acetonitrile to insure that all adsorbed SDS was eliminated. Column aging and surfactant desorption were checked measuring the efficiency obtained on the separation of the test mixture toluene + naphthalene with the 70% acetonitrile mobile phase [20].

3. Results and discussion

3.1. Retention behavior and apparent octanol–water partition coefficients

The acid–base dissociation constants in aqueous solution (pK_a) and molecular octanol–water partition coefficients (log $P^0_{o/w}$) of diuretics are given in Table 1. Only spironolactone is not an ionizable compound since it does not show acid–base behavior, whereas the other diuretics ionized at different pH values. These compounds can be classified in three groups: basic of the BH^+-B form (triamterene), weakly acidic (bendroflumethiazide, acetazolamide, trichloromethiazide, hydrochlorothiazide, chlorotiazide and benzthiazide), and acidic (xipamide, piretanide, furosemide, bumetanide, ethacrynic acid and probenecid), the last two groups belonging to the AH-A^- form. Dissociation constants are displaced in aqueous–organic and SDS micellar media compared to the aqueous solution values. The pH region where the molecular form of ionizable compounds dominates is extended. For acidic compounds of the AH-A^- type, the region where protonated AH species dominate is shifted towards higher pHs. The apparent constants have been determined for only a few diuretics. For instance, the pK_a values of furosemide and trichloromethiazide, are 3.8 and 7.3 in aqueous medium. In 30, 40 and 50% (v/v) acetonitrile solutions, these pK_a values increase to 4.8, 5.0 and 5.4, and 7.9, 8.3 and 8.8, respectively [39,40]. A ∼1 unit positive pH shift is also likely with SDS micellar phases [20].

In the aqueous–organic RPLC mode, the diuretics were eluted with aqueous–organic mobile phases with acetonitrile contents in the 30–50% range to avoid extremely long or short retention times. In the micellar mode, acetonitrile ranged between 10 and 20%, and SDS between 0.05 and 0.15 M. The intrinsic retentions of the acid form and the base form of ionizable compounds are completely different. This yields a sharp change in retention times at pH values close to the logarithm of the dissociation constant in the mobile phase medium. However, in the working pH range of the chromatographic column (3–7), this change can only be observed for the acidic diuretics. It was not observed for triamterene, the only BH^+-B basic compound, which means that its pK_a value (6.2) may also have been shifted toward a higher pH value.

Fig. 1 shows the dependence of retention factors with pH for the acidic diuretics eluted with a mobile phase of acetonitrile water and acetonitrile–SDS. In both chromatographic modes, the retention of weakly acidic diuretics (not shown) was constant or nearly constant in the full working pH range of the column, or slightly decreased at pH close to 7. The basic diuretic triamterene, which is positively charged in acidic and neutral medium, showed an expected reverse behavior: in aqueous–organic RPLC its retention increased with pH due to the greater affinity of the neutral species towards the stationary phase, whereas, in MLC, its retention decreased due to the decrease of the cationic protonated solute concentration produced by pH increases. This cationic form is highly retained in MLC with SDS because it is sensitive to electrostatic interactions with the stationary phase covered by adsorbed surfactant anions.

Retention factors of the ionizable compounds in 30% acetonitrile mobile phases ranged between $k = 1.2$ (acetazolamide) and 74 (bumetanide) at pH 3, and between 0.9 (acetazolamide) and 36.7 (bendroflumethiazide) at pH 7. In 50% acetonitrile, k ranged between 0.7 (acetazolamide) and 6.8 (ethacrynic acid) at pH 3, and between 0.6 (acetazolamide) and 4.2 (bendroflumethiazide) at pH 7. The neutral diuretic spironolactone showed always the largest retention. In MLC, k ranged between 0.6 and 57.3 at pH 3, and between 0.2 and 41.0 at pH 7, for the mobile phase containing only 10% acetonitrile (and 0.05 M SDS), and between 0.4 and 12.5 at pH 3, and between 0.1 and 6.7 at pH 7, for the mobile phase with the largest elution strength (0.15 M SDS–20% acetonitrile). Clearly, considering elution strength, there is a synergistic effect between micelles and acetonitrile. The presence of micelles in the mobile phase allows to save more than 60% of the organic modifier volume [20]. Minimal and maximal retention in MLC corresponded always to chlorothiazide and triamterene, respectively.

pH variations produce changes in the concentration ratio of the acid and base forms of ionizable compounds. These variations produce changes in the measured $P_{o/w}$ since the ionized form is much more polar or hydrophilic than the molecular form. This means that the measured $P_{o/w}$ have a conditional or apparent character (P_{app}). log P_{app} for the diuretics in the pH range 3–7 were calculated with Eqs. (1) and (2), using $P^0_{o/w}$, $P^-_{o/w}$ and $P^+_{o/w}$ values obtained in purely aqueous phase in a previous work using the CCC method [16], and pK_a in aqueous medium (Table 1). The calculated log P_{app} ranged between −1.54 (triamterene) and 2.18 (xipamide) at pH 3, and between −1.31 (furosemide) and 1.67 (benzthiazide) at pH 7. log $P_{o/w}$ for the neutral diuretic spironolactone was 2.73.

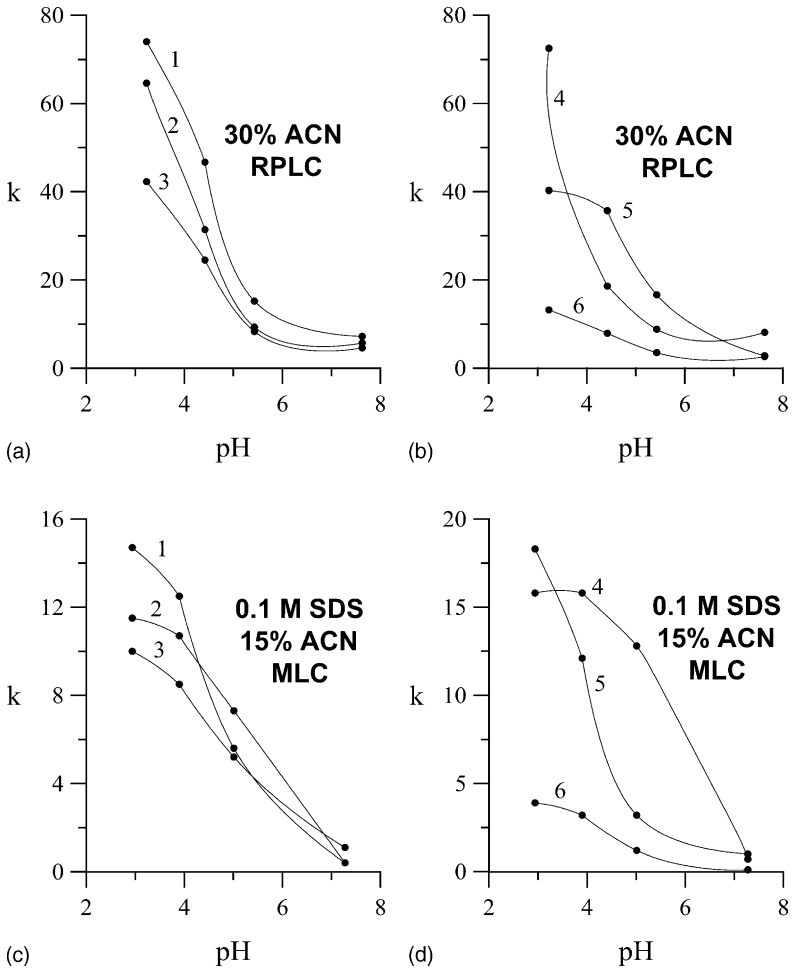


Fig. 1. Effect of pH on the retention of several diuretics in aqueous–organic RPLC (a, b) and MLC (c, d): (1) bumetanide, (2) probenecid, (3) piretanide, (4) ethacrynic acid, (5) xipamide, and (6) furosemide. Mobile phase composition: (a, b) 30% acetonitrile, and (c, d) 15% acetonitrile 0.10 M SDS.

3.2. Relationships between chromatographic retention and octanol–water partition coefficients

3.2.1. Aqueous–organic RPLC

Retention data (expressed as $\log k$) of diuretics in aqueous–organic mobile phases were plotted against $\log P^0_{o/w}$ (octanol–water partition coefficient for the molecular species) or $\log P_{app}$ (apparent value that considers the distribution of the acid–base pair), at several pH values, in order to examine the quality of the correlations between retention and hydrophobicity. The correlation coefficients are listed in Table 2. As can be seen, correlations with $\log P^0_{o/w}$ were very poor, but satisfactory when the apparent values were used instead in the pH range 3–5. The goodness of the correlations can be also observed in Fig. 2.

Retention decreased at increasing acetonitrile concentration and pH, and accordingly, the slope of the fitted $\log k$ versus $\log P_{app}$ straight-lines. For a single pH, the correlations were similar or no clear trend was observed at varying acetonitrile concentration. We should remind that all studied solutes, but triamterene and spironolactone, were acidic of the AH-A^- type. In a previous work [41], we studied a group of basic antihistamines of the BH^+-B type eluted

Table 2
Correlation coefficients of the $\log k$ vs. $\log P^0_{o/w}$ (molecular coefficients) or vs. $\log P_{app}$ (apparent partition coefficients) lines in aqueous–organic RPLC

$\log k_{acetonitrile\ (v/v)}$	$\log P^0_{o/w}$				$\log P_{app}$			
	pH 3	pH 4	pH 5	pH 7	pH 3	pH 4	pH 5	pH 7
$\log k_{30}$	0.875	0.865	0.783	0.742	0.938	0.983	0.943	0.725
$\log k_{40}$	0.855	0.833	0.683	0.591	0.939	0.982	0.956	0.819
$\log k_{50}$	0.840	0.800	0.618	0.489	0.933	0.976	0.946	0.852

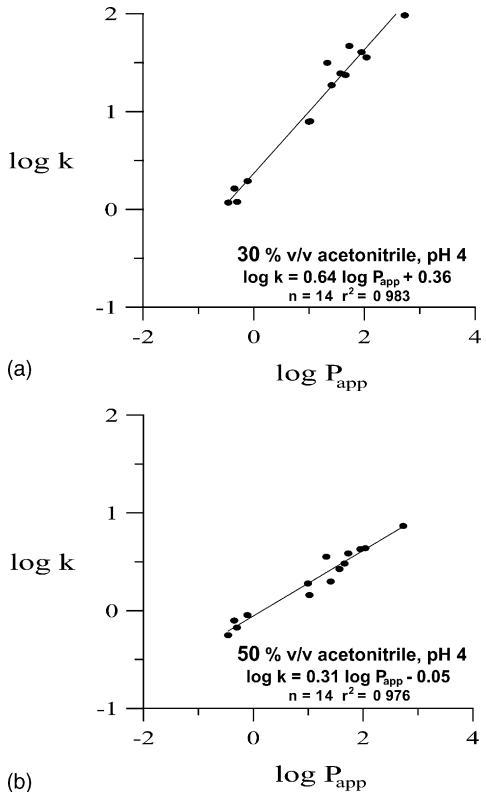


Fig. 2. Correlation between $\log k$ and $\log P_{app}$ for 14 diuretics eluted with aqueous–organic mobile phases at pH 4: (a) 30% acetonitrile, and (b) 50% acetonitrile.

with methanol–water mobile phases. We found the opposite trend: the regression coefficients for these correlations increased with the concentration of organic solvent in the mobile phase.

The quality of the correlations was optimal at pH 4 (Table 2). The relatively high correlation coefficients achieved in the $\log k$ versus $\log P_{app}$ plots at different concentrations of organic solvent and low pH, suggests that the main force that governs retention in this system is hydrophobicity. At pH 7 where ionic species of most diuretics dominate, correlations were poorer due to pK_a shifts, interactions with free silanols on the column, and/or to the low retention achieved in these conditions that made adequate measurement of retention times difficult. Correlations would improve by using corrected values of pK_a in the aqueous–organic media of the mobile phases instead of the values in aqueous medium. However, corrected constants were only available for ethacrynic acid, furosemide, bumetanide, chlorothiazide and trichlormethiazide [39,40,42]. For these diuretics, the pK_a values were 4.0, 4.8, 5.0, 7.1 and 7.9 in 30% acetonitrile, against 3.5, 3.8, 3.6, 6.7 and 7.3 in aqueous medium, respectively. The positive pK_a shifts were respectively 0.5, 1, 1.4, 0.4 and 0.6.

3.2.2. Micellar–organic RPLC

A similar study was made with retention data obtained in mixed mobile phases of acetonitrile and SDS. The results are given in Table 3. For this chromatographic mode, the correlations established for $\log k$ versus $\log P^0_{o/w}$ and $\log k$ versus $\log P_{app}$ were similar. In the presence of micelles, the acid–base equilibriums of our set of compounds, except triamterene, are shifted in a larger extent towards higher pH with respect to the aqueous–organic medium. Therefore, the region dominated by the non-ionic species increases. The correlations were poorer than those in aqueous–organic RPLC and deteriorated at larger micelle concentration. Fig. 3a shows the plot for 10% acetonitrile–0.05 M SDS at pH 4. Several authors have claimed the improvement in the correlations in MLC when k is used instead of $\log k$ [35,36,43]. This improvement was not observed in the case of the diuretic compound studied.

The retention data of the basic diuretic triamterene were excluded from these correlations, since it belongs to the BH^+-B type. Retention for this drug was larger than expected according to its hydrophobicity measured as $\log P_{app}$. The increased retention is produced by the additional electrostatic interaction established between the positively charged species and the column modified by the adsorption of monomers of the anionic surfactant. Both electrostatic and hydrophobic interactions are thus combined for this compound. This behavior has always been observed for compounds of the BH^+-B type such as antihistamines [41], the diuretic amiloride and several β-blockers [44], phenethylamines [45], and tricyclic antidepressants [46].

3.3. Relationships with isocratic descriptors

3.3.1. Aqueous–organic RPLC

A parameter independent of the concentration of organic modifier in the mobile phase is believed to be more useful for hydrophobicity correlation studies. In this sense, $\log k_w$,

Table 3
Correlation coefficients of the $\log k$ vs. $\log P^0_{o/w}$ (molecular coefficients) or vs. $\log P_{app}$ (apparent partition coefficients) lines with micellar phases in MLC

$\log k_{\text{acetonitrile (v/v)–SDS (M)}}$	$\log P^0_{o/w}$				$\log P_{app}$			
	pH 3	pH 4	pH 5	pH 7	pH 3	pH 4	pH 5	pH 7
$\log k_{10–0.05}$	0.930	0.935	0.905	0.454	0.928	0.899	0.840	0.948
$\log k_{20–0.05}$	0.928	0.936	0.838	0.405	0.927	0.923	0.933	0.944
$\log k_{15–0.10}$	0.919	0.897	0.890	0.470	0.918	0.893	0.846	0.931
$\log k_{10–0.15}$	0.907	0.911	0.899	0.472	0.904	0.866	0.762	0.885
$\log k_{20–0.15}$	0.910	0.919	0.877	0.281	0.909	0.890	0.853	0.911

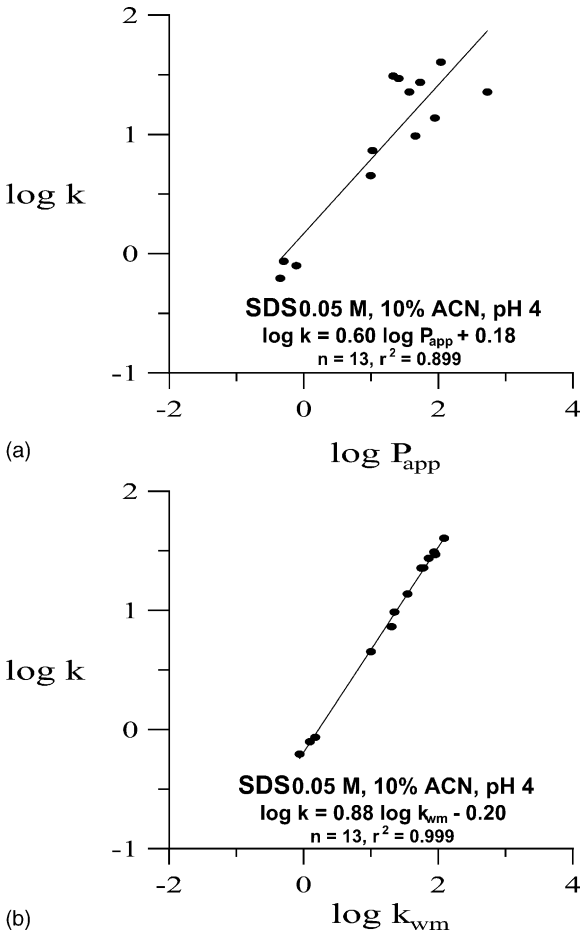


Fig. 3. Correlations between: (a) $\log k$ and $\log P_{app}$, and (b) $\log k$ and $\log k_{wm}$ for 13 diuretics (triamterene was excluded), eluted with micellar mobile phases. Mobile phase composition for $\log k$: 10% acetonitrile–0.05 M SDS at pH 4.

an estimation of the retention factor at null concentration of organic modifier (i.e. pure water), is one of the most widely used chromatographic descriptors of hydrophobicity [3–11], despite being an extrapolated value that can yield large errors and different results for different modifiers. The extrapolated $\log k_w$ is calculated from the plot of $\log k$ for each compound versus organic modifier, since the chromatographic retention in binary aqueous–organic mobile phases can be modeled as a linear function:

$$\log k = \log k_w + S\varphi \quad (3)$$

where $\log k$ is the solute retention factor at a specific mobile phase composition and φ the volumetric fraction of organic solvent; S is an estimation of the mobile phase elution strength.

$\log k$ data follow a parabolic behavior for wide ranges of organic modifier. This can give problems in the extrapolation to pure water when plotting $\log k$ versus φ. For diuretics in the working acetonitrile range, the fits were however linear at all pH values examined ($r > 0.99$, except for some compounds at pH 7 with $r \approx 0.98$).

Retention data for aqueous–organic mobile phases were plotted at constant pH value versus $\log k_w$. As observed in Table 4, correlations were satisfactory in acidic medium and deteriorated at increasing pH and larger acetonitrile content, where retention was smaller. Fig. 4a shows the plot obtained at pH 4 for 30% acetonitrile.

The chromatographic hydrophobicity index, φ_0, has been introduced recently [4,6]. This descriptor is defined as the volume percentage of organic solvent in the mobile phase producing a retention factor of unity, that is the retention time is twice the dead time and $\log k = 0$. The φ_0 values are obtained from the intercept ($\log k_w$) and the slope of the straight line plots of $\log k$ versus organic solvent in the mobile phase. From Eq. (3), φ_0 is simply obtained as:

$$\varphi_0 = -\frac{\log k_w}{S} \quad (4)$$

Reasonably good correlation coefficients (Table 4) were achieved for $\log k$ versus φ_0 in the pH range 3–5 ($r = 0.93$–0.95). The correlation deteriorated at increasing pH, but no clear dependence was observed with respect to the acetonitrile content. However, the correlations were significantly poorer with φ_o than those obtained with $\log k_w$ Fig. 4b illustrates the dependence of $\log k$ with φ_0 for 30% acetonitrile at pH 4. It seems that the S parameter is

Table 4
Correlation coefficients between retention data and isocratic descriptors in aqueous–organic RPLC mobile phases and micellar–organic MLC mobile phases

	pH 3			pH 4			pH 5			pH 7		
	$\log k_w$	φ_0	$\log k_{wm}$	$\log k_w$	φ_0	$\log k_{wm}$	$\log k_w$	φ_0	$\log k_{wm}$	$\log k_w$	φ_0	$\log k_{wm}$
$\log k_{\text{acetonitrile (v/v)}}$												
$\log k_{30}$	0.998	0.948		0.997	0.944		0.992	0.937		0.982	0.889	
$\log k_{40}$	0.993	0.957		0.986	0.954		0.950	0.939		0.899	0.884	
$\log k_{50}$	0.986	0.956		0.970	0.951		0.914	0.932		0.819	0.851	
$\log k_{\text{acetonitrile (v/v)–SDS (M)}}$												
$\log k_{10–0.05}$			0.999			0.999			0.996			0.932
$\log k_{20–0.05}$			0.991			0.988			0.939			0.868
$\log k_{15–0.10}$			0.997			0.998			0.992			0.925
$\log k_{10–0.15}$			0.995			0.996			0.995			0.956
$\log k_{20–0.15}$			0.993			0.994			0.986			0.707

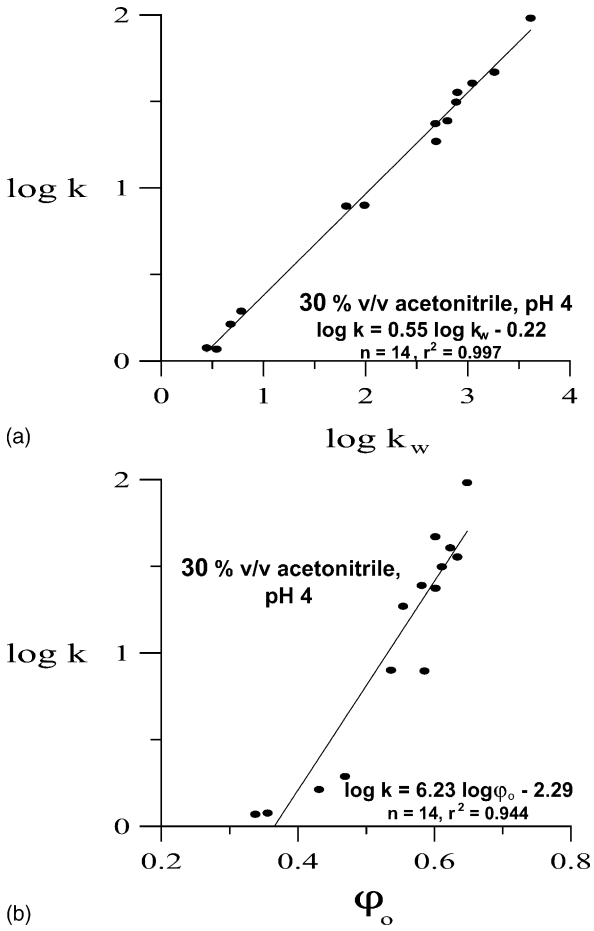


Fig. 4. Correlations between: (a) $\log k$ and $\log k_w$, and (b) $\log k$ and φ_0, for 14 diuretics eluted with aqueous–organic mobile phases. Mobile phase composition for $\log k$: 30% acetonitrile at pH 4.

correlated to hydrophobicity and to several other physicochemical properties.

3.3.2. Micellar–organic RPLC

As for classical RPLC, a parameter independent of the value of the two main variables in MLC (surfactant and organic modifier concentrations) should also be useful in correlation studies. Similarly to $\log k_w$, a parameter k_{wm} was defined as the retention factor extrapolated to a mobile phase with nil micelle and organic solvent concentrations. In previous work, a theoretical model proposed by Strasters et al. [47] (Eq. (5)) was used for a group of β-blockers of the BH^+-B type and SDS anionic micelles [25]:

$$\log k = \log k_{wm} + S\varphi + S' [M] \quad (5)$$

Eq. (5) was applied to diuretics of the AH-A^- type, giving rise to linear plots with correlation coefficients in the range $r = 0.98$–0.99 at pH 3–4, which deteriorated at increasing pHs (especially at pH 7). $\log k$ correlated also satisfactorily with $\log k_{wm}$ values (Table 4). The correlations were excellent at acidic pH for any studied mobile phase composition ($r = 0.98$–0.99), being significantly better than

Table 5
Correlation coefficients between isocratic descriptors and apparent partition coefficients for RPLC and MLC mobile phases

	pH 3		pH 4		pH 5	
	$\log P_{app}$	$\log k_w$	$\log P_{app}$	$\log k_w$	$\log P_{app}$	$\log k_w$
$\log k_w$	0.936	1	0.976	1	0.917	1
φ_0	0.940	0.941	0.938	0.933	0.852	0.914
$\log k_{wm}$	0.931	0.965	0.891	0.936	0.803	0.884

for the aqueous–organic mobile phases. Fig. 3b depicts the plot for 10% acetonitrile–0.05 M SDS at pH 4, it should be compared with Fig. 4a (30% acetonitrile).

The isocratic descriptors $\log k_w$, φ_0 and $\log k_{wm}$ were next correlated among them and with $\log P_{app}$. As expected, good results were only obtained at acidic pH. The results are given in Table 5. The three descriptors showed acceptable correlations with $\log P_{app}$ at pH 3 and 4. When compared with $\log k$ versus $\log P_{app}$, the correlations were poorer for aqueous–organic RPLC and significantly better for MLC. Fig. 5a shows the $\log k_w$ versus $\log P_{app}$ plot for acetonitrile water mixtures at pH 4. The slope is close to unity.

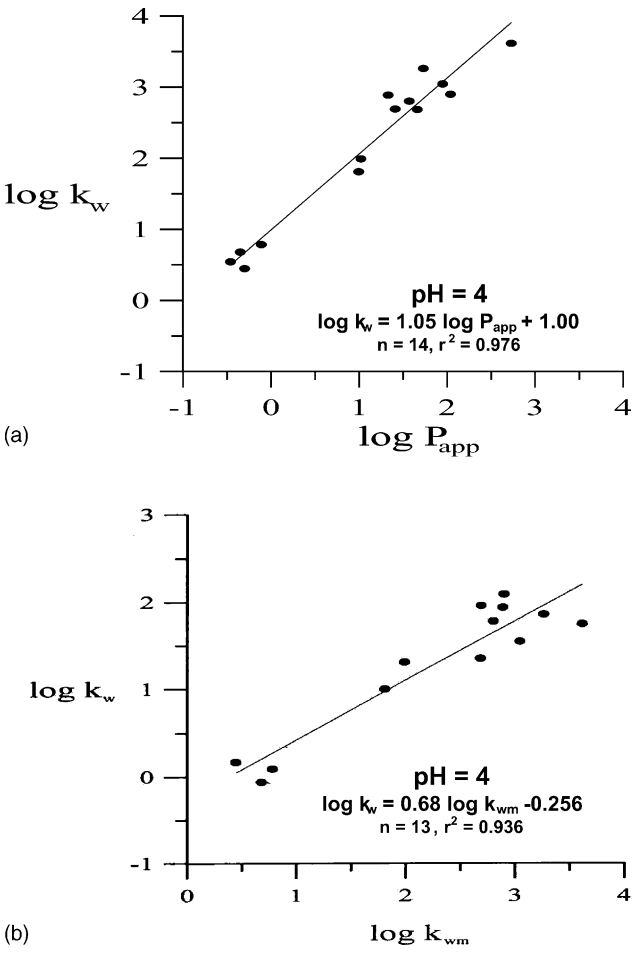


Fig. 5. Correlations between: (a) $\log k_w$ and $\log P_{app}$, and (b) $\log k_w$ and $\log k_{wm}$, at pH 4.

Finally, the micellar chromatographic index $\log k_{wm}$ was observed to correlate satisfactorily with its equivalent aqueous–organic descriptor $\log k_w$ at pH 3 and 4 (Table 5 and Fig. 5b). Both parameters, k_w and k_{wm} correspond to extrapolations of retention to pure water (absence of modifiers). They should be identical. They are not. This result was already observed and commented in the literature were comments on the achievement of different k_w for acetonitrile and methanol in classical RPLC are usual [4,7]. However, interestingly, correlation equations obtained at pH 3 and 4 were similar: $\log k_w = 0.65 \log k_{wm} - 0.243$ ($n = 13$, $r^2 = 0.965$) and $\log k_w = 0.68 \log k_{wm} - 0.256$ ($n = 13$, $r^2 = 0.936$), respectively.

4. Conclusions

When working with ionizable compounds, it is extremely important to know the octanol–water partition coefficient of the molecule, the coefficient of the ionized form, either anionic or cationic, and the dissociation constant. The latter parameter may be significantly shifted in aqueous–organic phases as well as in micellar solutions. Retention data ($\log k$) of 14 diuretics mainly of the AH-A^- type in the working pH range of a conventional octadecylsilane column (pH 3–7), and the isocratic descriptors $\log k_w$ and φ_0 for aqueous–organic phases and k_{wm} for SDS micellar–organic solutions, were correlated with molecular and apparent octanol–water partition coefficients at several pH values using the aqueous dissociation constants. The results will seemingly improve using the dissociation constants in the acetonitrile water and acetonitrile–SDS mixtures. These constants may differ from the aqueous phase value by one unit or more. They are now only available for few proportions of aqueous–organic media and SDS micellar phases and few diuretics.

With aqueous–organic RPLC chromatographic data, the quality of the correlations obtained for diuretics with the handicap of using aqueous dissociation constants appears to confirm that $\log P_{app}$ at varying ionization degree is effective when establishing correlations. In the very limited cases studied, the ionization correction seems to be less effective with SDS micelles probably due to the larger shift of acid–base equilibria towards higher pH for the AH-A^- ionizable compounds. The correlations were also satisfactory for the plots of $\log k_w$ and $\log k_{wm}$ versus $\log P_{app}$, although poorer than those established for $\log k$. The extrapolation used to obtain the $\log k_w$ and $\log k_{wm}$ values introduced a cumulative error. These parameters have however the great advantage to be independent on the modifier concentration.

In aqueous–organic RPLC, retention depends mainly on hydrophobicity, although there is some contribution of the interaction of ionic species and hydrogen-bond acidity with free silanols on the column [10]. A strong electrostatic interaction between solutes and the stationary phase can take place in MLC with anionic surfactant, where the stationary phase is negatively charged due to surfactant adsorption. The anionic species formed by dissociation of acidic solutes are repelled, which may explain, at least partially, the poorer correlations with hydrophobicity at neutral pH. On the other hand, protonated basic compounds are strongly attracted to the stationary phase and their retention is appreciably larger than expected from hydrophobicity. In the case of the few ionizable diuretics studied, classical RPLC seems to be superior to MLC with SDS micelles for hydrophobicity studies.

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